STUDIES ON THE SEASONAL CHANGES IN THE CHEMICAL CONSTITUENTS OF THE PEARL OYSTER*

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With 8 Text-figures and 10 Tables

Not only in morphology but also in metabolism many marked differences have been observed between Invertebrata and Vertebrata. Especially differences in the chemical process of calcification are typical. The mechanism of shell formation by the mollusks, however, has not yet been so thoroughly studied as the process of bone formation.

Although a sort of pearls is produced by many kinds of bivalved mollusks, that of the pearl oyster, *Pinctada martensii*, is said to be superior. In the culture pearl industry of Japan, this kind of mollusk has been used almost exclusively (1) (2).

It may be expected that the quality of the pearl formed by the pearl oyster is influenced by various external conditions under which the pearl oysters have been cultivated. For several years the process of calcification in the pearl oyster has been studied from the biochemical standpoint at this laboratory (3). The seasonal variations in the chemical constituents of the pearl oyster were first investigated. Especially, since the inorganic constituents are presumed to have close relation to the formation of the pearl and of the shell, their seasonal changes were precisely investigated.

EXPERIMENTAL

Materials

The pearl oysters used in these studies were two- or three-winter mollusks cultivated at the beach of The Seto Marine Biological Laboratory of The Kyoto University at Shirahama Town in Wakayama Prefecture.

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Analysis were performed once a month from October to the following September. For the determination of glycogen content several oysters were treated at The Marine Laboratory and the rest were brought to The Biochemical Laboratory of The Kyoto University and a measurement of other constituents was conducted.

Methods

To determine the total weight, fresh meat and dried meat, water content, total nitrogen, lipids, glycogen and ash, fifteen oysters were used each time. The analysis of each constituent was carried out as follows (4).

Water (*Moisture*) and *ash:* Adductor muscles were separated from other parts and were dried at room temperature between filter papers. Water content was estimated from the decrease of weight after drying the meat at 105° to 110°C. in the oven. Dried meat was burnt to ashes in the muffle.

Total nitrogen (5): Total nitrogen was determined by KJELDAHL's method using metallic selenium as completely oxidizing catalyst.

Lipids: Lipids were extracted with ether by using SOXHLET's extractor for about sixteen hours from the dried meat, dehydrated in vacuum at 40°–45°C. for about six hours.

Glycogen (6): Since glycogen content in the pearl oyster is reported to be variable during the day (7), six oysters were taken up from the sea at 9 a.m. each time for the determination of glycogen content. As soon as possible glycogen was isolated from the fresh meats by means of PFLEGER's method and was hydrolysed with HCl. The amounts of glucose thus formed were determined by FEHLING-LEHMANN-SHOORL's method and the glycogen content was calculated.

Inorganic constituents (8): For the determination of the inorganic constituents, ashes from the dried meats of thirty oysters were used. The ash was treated with HCl and after filtrating the trace amount of silica separated, phosphate was precipitated by ammonium molybdate. From this filterate iron and aluminium were precipitated by the addition of ammonia water. Calcium and magnesium were determined as oxalate and phosphate respectively with the filtrate from iron. With the solution free from phosphate, sulphate, magnesium, iron, aluminium and manganese by baryta, the determination of alkali metals was carried out by the platinium chloride method and also by the magnesium uranyl acetate method. Sulphate was measured as barium sulphate and chloride was also determined by the silver chloride method.

RESULTS AND DISCUSSION

Seasonal changes of the average values of total weight, of weight of the shell, of the fresh meat and of the dried meat, and content of water per oyster are shown in Table I and Fig. 1.

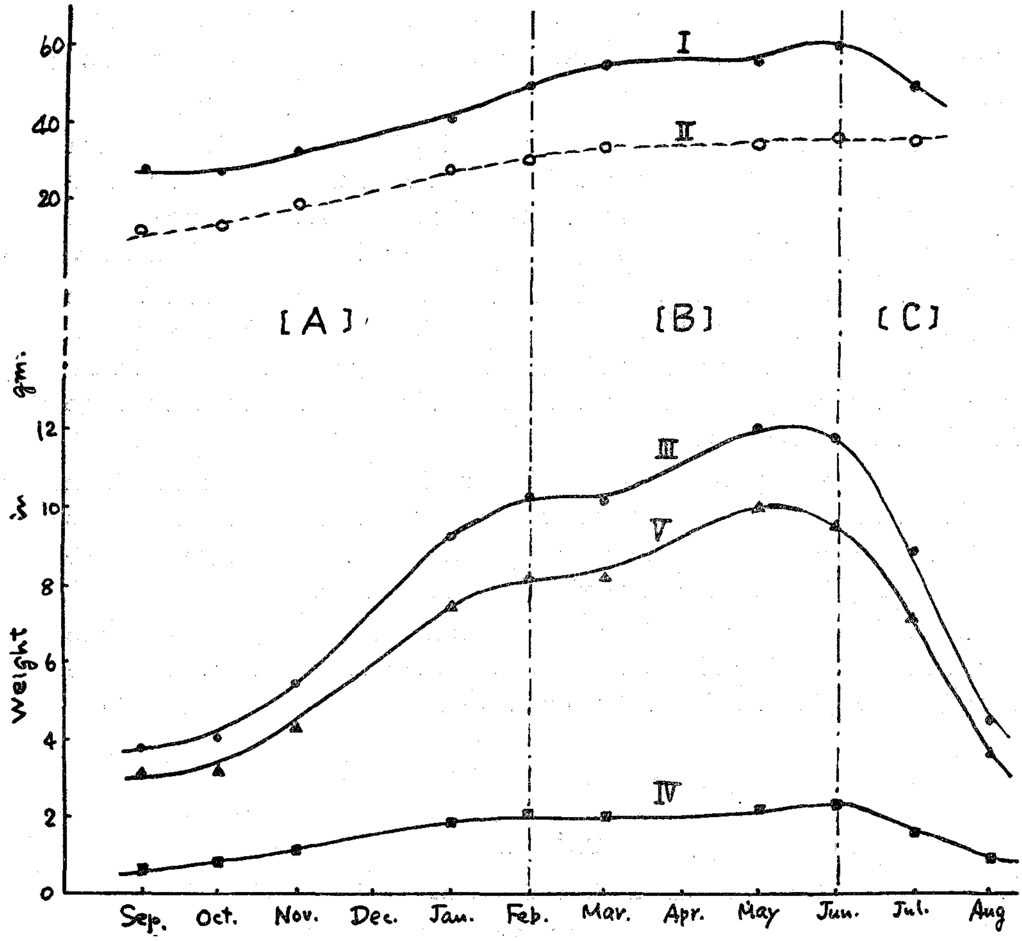

Fig. 1. The Monthly Changes in the Weight of the Pearl Oyster (per a Pearl Oyster). Curve I: Total weight, II: Shell weight, III: Fresh meat weight, IV: Dried meat weight and V: Water content. Period [A]: The fattening period, [B]: The ripening period and [C]: The reproductive period.

Table I.
The Monthly Changes in the Weight of the Pearl Oyster
(per a Pearl Oyster).

	Sep.	Oct.	Nov.	Jan.	Feb.	Mar.	May	Jun.	Jul.
	g	g	g	g	g	g	g	g	g
Total weight	27.0	26.4	33.6	41.5	50.4	56.5	56.0	61.5	51.0
Shell weight	13.1	12.4	18.6	28.6	30.7	34.7	34.8	38.1	35.7
Fresh meat weight	3.83	3.98	54.8	9.31	10.28	10.10	12.11	11.36	8.84
Dried meat weight	0.64	0.77	1.14	1.78	2.05	2.00	2.17	2.32	1.60
Water content	3.18	3.22	4.34	7.53	8.23	8.10	9.95	9.55	7.24

Remarkable increases in total weight and weight of the shell through out these experiments were observed. Fresh and dried meat increased gradually from September to the following June in weight. A sudden decrease to the extent of about half of weight in May was observed to occur in summer.

From these facts, the season from autumn to winter is presumed to be the fattening period of the pearl oyster, which in spring the vegetative growth ceases and the gonads gradually ripen (the ripening period). The summer is the reproductive period, when gonads have fully ripened and the discharge of the sexual products occurs.

It is noteworthy that in spite of decrease in the weight of the fresh meat, dried meat increases in weight during May and June. This fact shows that hydrolysis or other chemical reactions which need water may take place in the oyster before the discharge of the sexual products.

The monthly changes in the chemical constituents of the pearl oyster are shown in Table II–VII and Fig. 2–6. Those of the adductor muscle alone are shown in Table V and VII and Fig. 5 and 6.

Table II.

The Monthly Changes in the Chemical Constituents of the Pearl Oyster (in Weight).

	Sep.	Oct.	Nov.	Jan.	Feb.	Mar.	May	Jun.	Jul.	Aug.
	g	g	g	g	g	g	g	g	g	g
Total nitrogen	0.080	0.078	0.130	0.200	0.208	0.222	0.233	0.262	0.188	0.105
Crude protein	0.500	0.487	0.810	1.246	1.297	1.400	1.589	1.635	1.177	0.657
Lipids	0.059	0.084	0.102	0.191	0.241	0.110	0.094	0.113	0.058	0.073
Glycogen	0.003	0.033	0.079	0.150	0.176	0.264	0.150	0.163	0.110	0.011
Total ash	0.077	0.088	0.096	0.101	0.162	0.188	0.162	0.189	0.154	0.094

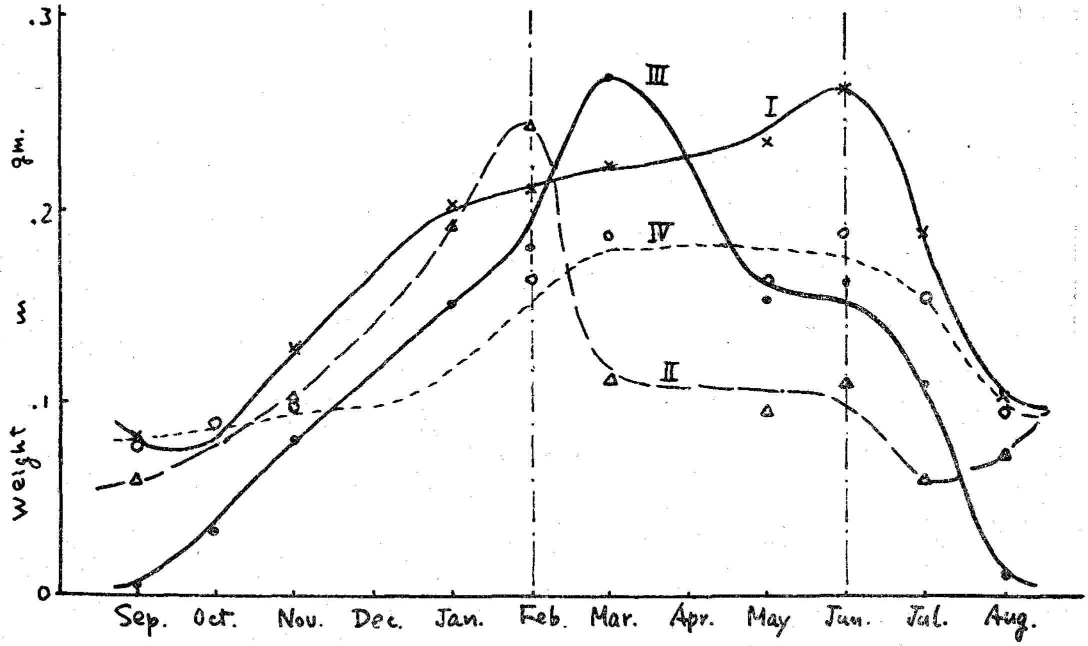

Fig. 2. The Monthly Changes in the Chemical Constituents of the Pearl Oyster (in Weight). Curve I: Total nitrogen, II: Lipids, III: Glycogen and IV: Total ash.

Table III.

The Monthly Changes in the Chemical Constituents of the Pearl Oyster

(in Percentage).

	Sep.	Oct.	Nov.	Jan.	Feb.	Mar.	May	Jun.	Jul.	Aug.
	%	%	%	%	%	%	%	%	%	%
Water	83.24	80.56	79.40	81.20	79.85	78.11	81.50	80.22	81.57	80.54
Dried meat	16.76	19.44	20.60	18.80	20.15	21.89	18.50	19.78	18.43	19.46
Total nitrogen	12.50	10.13	11.39	11.21	10.13	11.10	11.72	12.28	11.76	12.23
Crude protein	78.20	63.31	71.16	70.06	63.30	70.00	73.24	70.50	73.52	76.45
Lipids	9.20	10.90	8.91	10.70	11.77	5.48	4.35	4.88	3.60	8.52
Glycogen	0.40	3.93	6.90	8.40	8.57	13.18	8.91	7.02	6.87	1.24
Total ash	12.10	11.41	8.40	5.65	7.90	9.41	7.46	8.13	9.63	10.85

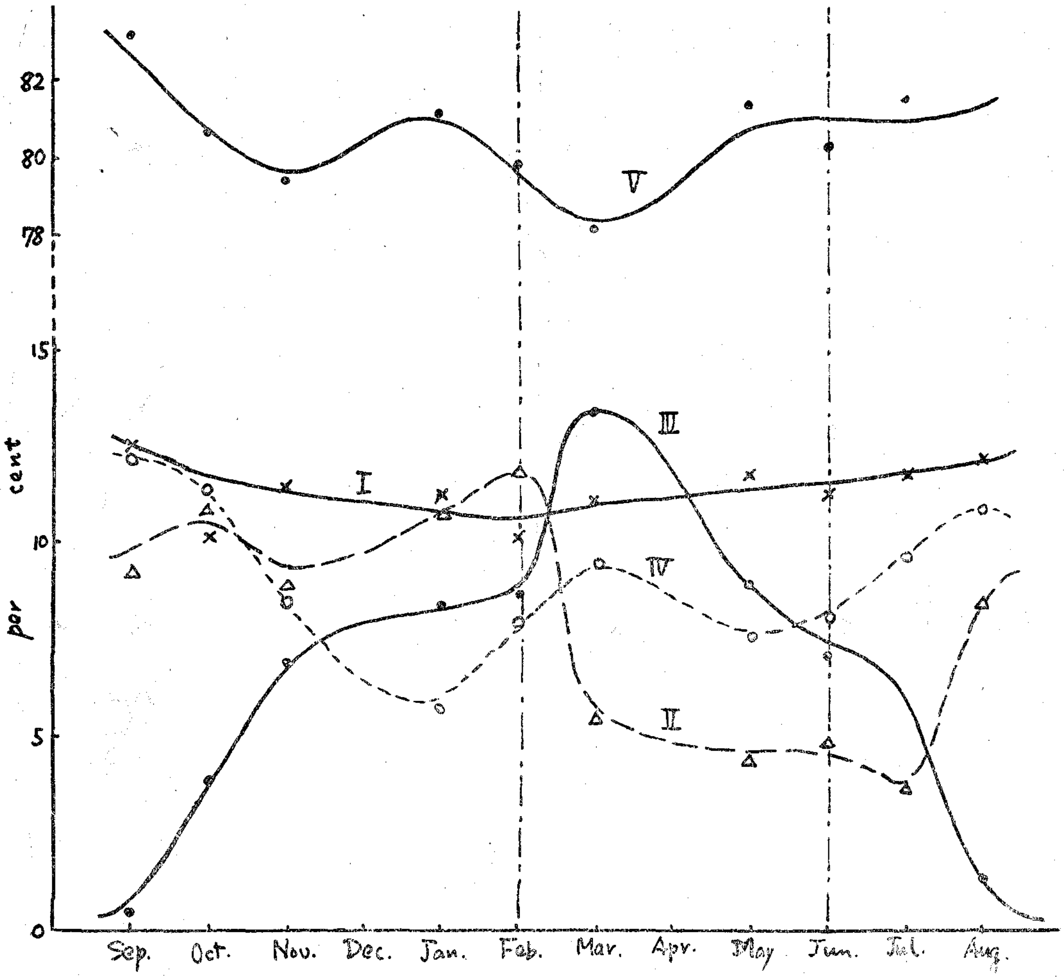

Fig. 3. The Monthly Changes in the Chemical Constituents of the Pearl Oyster (in Percentage). Curve I: Total nitrogen, II: Lipids, III: Glycogen and IV: Total ash (to Dried meat). Curve V: Water (to Fresh meat).

Table IV.

The Monthly Changes in the Chemical Constituents of Body without Adductor Muscle (in Weight).

	Nov.	Jan.	Feb.	Mar.	May	Jun.	Jul.	Aug.
	g	g	g	g	g	g	g	g
Fresh meat	3.90	6.76	7.20	7.15	8.54	8.82	6.70	3.42
Water	3.13	5.61	5.86	5.70	7.15	7.13	5.54	2.78
Dried meat	0.77	1.15	1.33	1.30	1.44	1.68	1.16	0.62
Total nitrogen	0.087	0.130	0.136	0.151	0.170	0.191	0.133	0.071

Crude protein	0.542	0.812	0.848	0.945	1.060	1.192	0.828	0.441
Lipids	0.081	0.127	0.157	0.104	0.096	0.111	0.059	0.065
Glycogen	0.037	0.093	0.106	0.073	0.059	0.034	0.041	0.004
Total ash	0.070	0.080	0.131	0.154	0.131	0.149	0.130	0.079

Table V.

The Monthly Changes in the Chemical Constituents of Adductor Muscle (in Weight).

	Nov.	Jan.	Feb.	Mar.	May	Jun.	Jul.	Aug.
	g	g	g	g	g	g	g	g
Fresh meat	1.58	2.55	3.08	2.95	3.57	3.04	2.14	1.07
Water	1.21	1.92	2.37	2.40	2.80	2.42	1.70	0.83
Dried meat	0.37	0.63	0.72	0.70	0.73	0.64	0.44	0.24
Total nitrogen	0.043	0.066	0.073	0.073	0.085	0.072	0.053	0.032
Crude protein	0.267	0.417	0.455	0.456	0.532	0.449	0.340	0.201
Lipids	0.021	0.061	0.022	0.020	0.017	0.019	0.009	0.010
Glycogen	0.040	0.057	0.081	0.171	0.084	0.127	0.061	0.007
Total ash	0.024	0.025	0.041	0.043	0.040	0.040	0.030	0.020

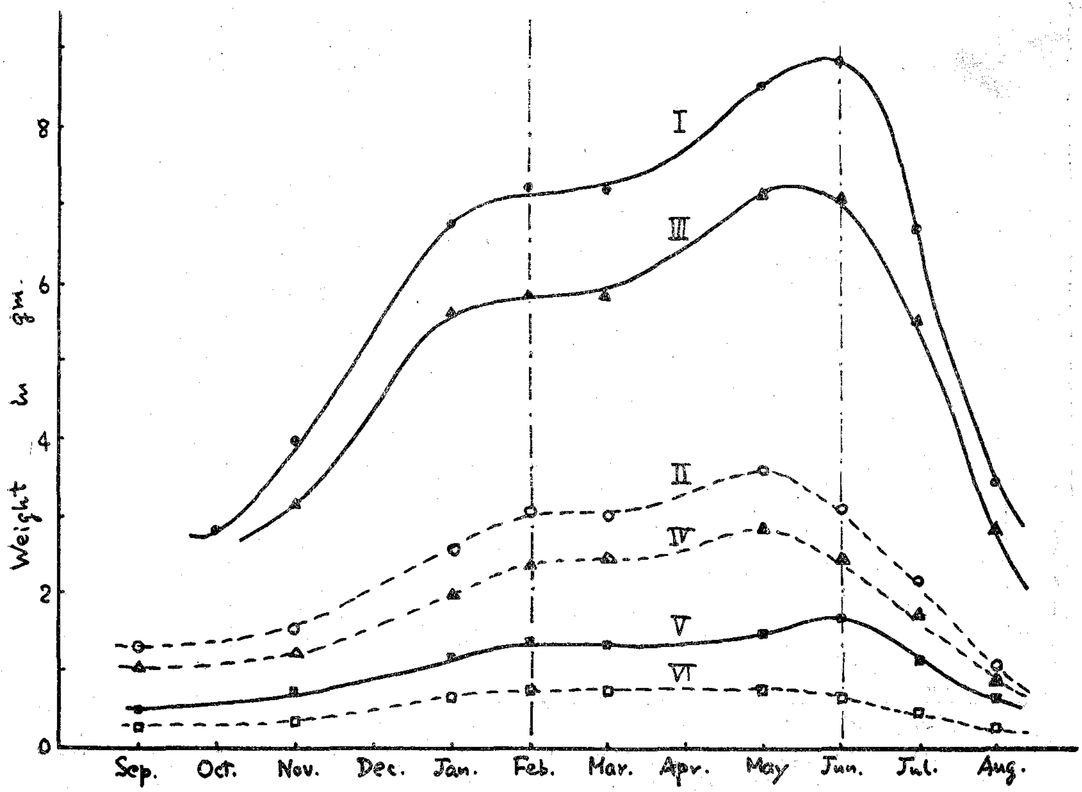

Fig. 4. The Monthly Changes in the Weight of Adductor Muscle and Total Other Parts of the Body of the Pearl Oyster. Curve I; Fresh meat in Body, II: Fresh meat in Adductor Muscle, III: Water in Body, IV: Water in Adductor Muscle, V: Dried meat in Body and VI; Dried meat in Adductor Muscle.

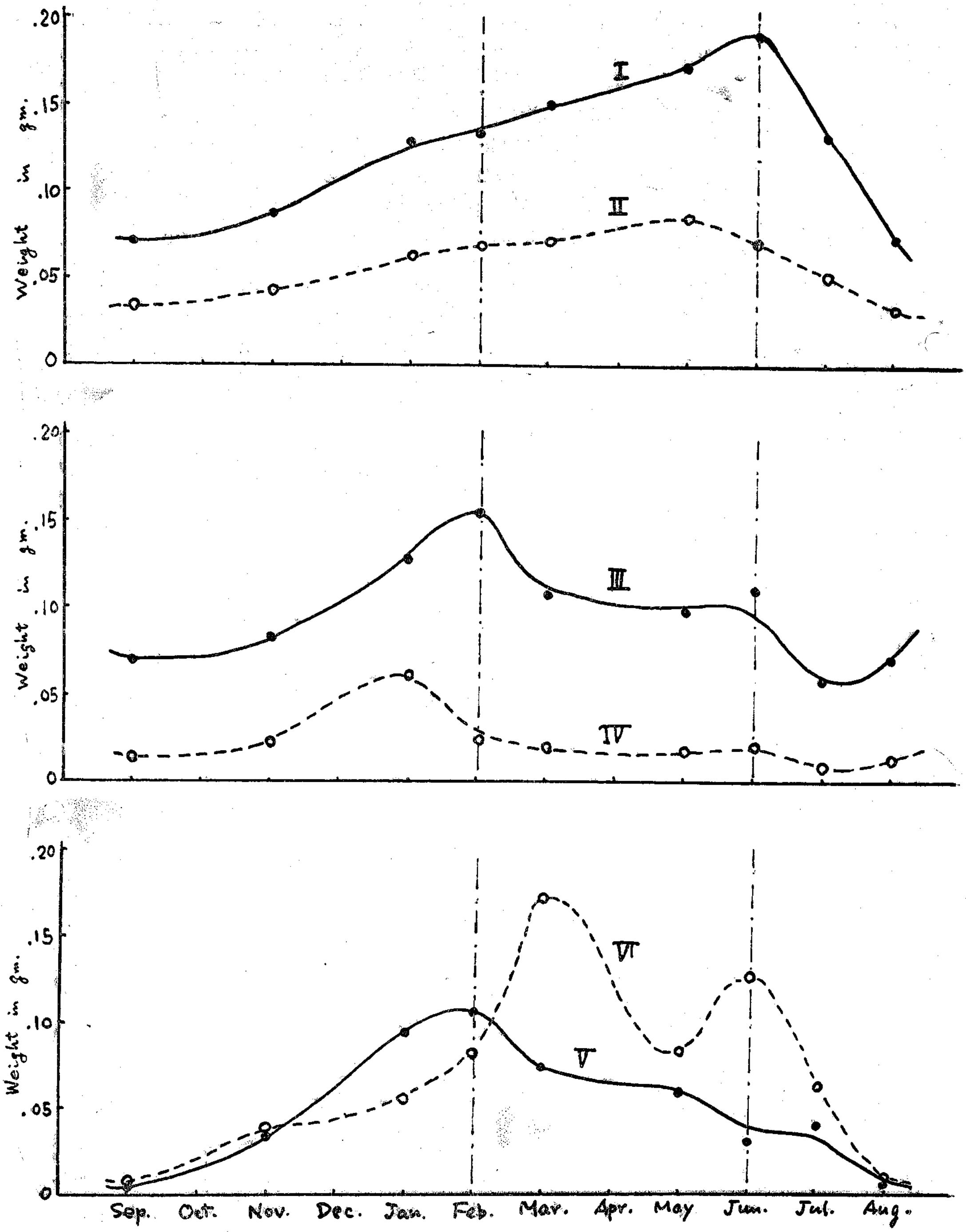

Fig. 5. The Monthly Changes in the Weight of Total Nitrogen, Lipids and Glycogen of the Adductor Muscle and Total Other Parts of the Body of the Pearl Oyster. Curve I; Total nitrogen in Body, II: Total nitrogen in Adductor Muscle, III: Lipids in Body, IV: Lipids in Adductor Muscle, V: Glycogen in Body and VI: Glycogen in Adductor Muscle.

Table VI.

The Monthly Changes in the Chemical Constituents of Body without Adductor Muscle (in Percentage).

	Nov.	Jan.	Feb.	Mar.	May	Jun.	Jul.	Aug.
	%	%	%	%	%	%	%	%
Water	80.22	82.95	81.44	80.65	83.05	80.86	82.69	81.88
Dried meat	19.78	17.04	18.56	19.34	16.95	19.14	17.31	18.12
Total nitrogen	11.26	11.30	10.20	11.63	11.78	11.36	11.43	11.38
Crude protein	70.40	70.63	63.75	72.65	73.60	71.00	71.40	71.15
Lipids	10.51	11.03	11.82	8.03	6.69	6.58	5.10	10.52
Glycogen	4.81	8.07	7.93	5.58	4.07	2.02	3.51	0.56
Total ash	9.04	6.99	9.84	11.87	9.11	8.85	11.19	12.67

Table VII.

The Monthly Changes in the Chemical Constituents of Adductor Muscle (in Percentage).

	Nov.	Jan.	Feb.	Mar.	May	Jun.	Jul.	Aug.
	%	%	%	%	%	%	%	%
Water	76.31	75.31	76.98	75.31	79.22	79.62	79.32	77.35
Dried meat	23.69	24.68	23.01	24.69	20.78	20.38	20.68	22.65
Total nitrogen	11.56	10.41	10.11	10.41	11.65	11.21	12.10	13.36
Crude protein	72.25	65.15	63.20	65.15	72.85	70.10	75.65	83.50
Lipids	5.63	9.59	2.98	2.83	2.27	3.01	2.01	4.31
Glycogen	10.93	8.95	11.29	24.38	11.45	19.82	13.81	3.05
Total ash	6.36	3.98	5.65	6.15	5.44	6.23	6.84	8.14

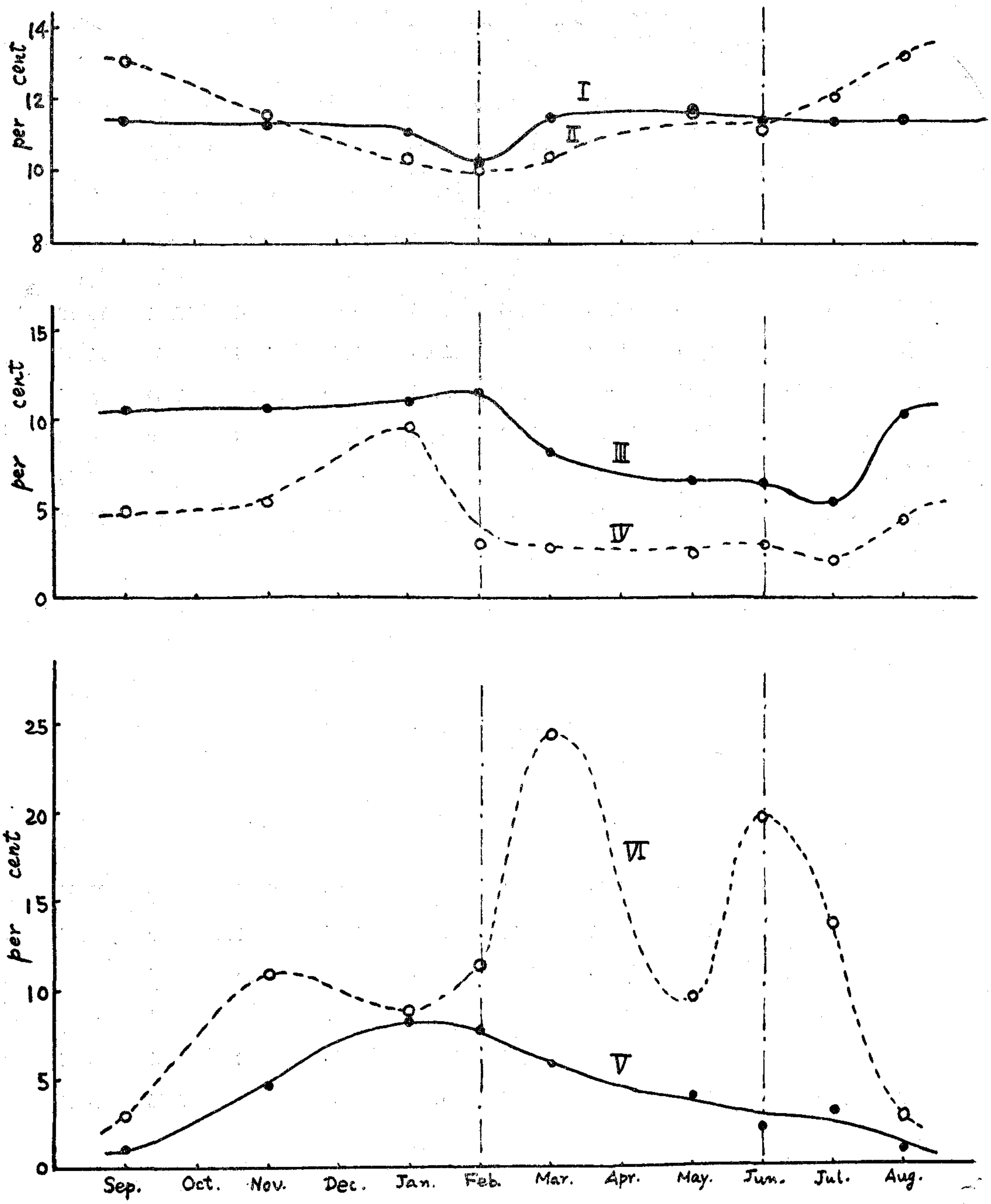

Fig. 6. The Monthly Changes in Percentage of Total Nitrogen, Lipids and Glycogen of the Adductor Muscle and Total Other Parts of the Body of the Pearl Oyster. Curve I: Total nitrogen in Body, II: Total nitrogen in Adductor Muscle, III: Lipids in Body, IV: Lipids in Adductor Muscle, V: Glycogen in Body and VI: Glycogen in Adductor Muscle.

Seasonal changes in protein content, which is the main component of the meat and of the adductor muscle, were found to be less striking than those occurring in other components, such as glycogen and lipids.

In the beginning of the fattening period the storage of glycogen is very remarkable. Notwithstanding that only trace glycogen was contained in September it increased rapidly and reached its maximum content (15 %) at the end of the fattening period. Especially in February glycogen was observed to be accumulated in the meat (except the adductor muscle) and then transferred into the adductor muscle in March. In contrast to the continuous increase in meat, glycogen begins to decrease gradually till May or June. From these facts certain active substances required for reproduction may be produced in this ripening period with the energy evolved from the oxidation of glycogen.

Lipids were also observed to be accumulated in the fattening period and reach maximum content in January in the adductor muscle and in February in the meat respectively. It is supposed from the facts that like glycogen lipids are utilized as sources of energy required for reproductive activities. The variation curves of lipids and glycogen suggest that transformations of lipids to glycogen may take place in the mollusk.

In contrast to the near-exhaustion of glycogen at the end of the ripening period, definite amounts of the lipids were noticed as still remaining. These constant lipids are presumed to have some physiological significance.

The results of analysis of the adductor muscle show that this organ serves not only to open and to shut the shell but also as a storage device of the oyster.

In contrast to the decrease of ash content a remarkable increase in shell weight was observed in the autumn. The season of pearl formation in the oyster is supposed to coincide with this season.

The seasonal changes in calcium, magnesium, iron, potassium, sodium, phosphate, chloride and sulphate content are shown in Table VIII and IX and also Fig. 7 and 8.

Table VIII.

The Monthly Changes in the Inorganic Constituents of the Pearl Oyster (in Weight).

	Sep.	Oct.	Nov.	Jan.	Feb.	Mar.	May	Jun.	Jul.	Aug.
	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg
P_2O_5	8.3	12.2	11.9	12.0	18.4	20.7	15.4	20.2	19.0	12.9
CaO	7.3	7.8	9.7	11.0	17.0	18.3	16.6	21.1	16.5	10.6
MgO	4.3	5.0	5.6	5.9	9.5	11.1	8.1	10.8	8.1	4.1
Fe_2O_3	1.2	1.2	1.6	2.0	4.1	6.2	4.2	4.3	3.5	1.4

K_2O	12.7	14.4	14.7	14.9	21.5	25.9	22.4	29.2	23.7	14.5
Na_2O	5.6	6.8	6.5	7.2	11.5	14.4	12.3	14.3	11.6	7.1
Cl	29.9	33.0	36.4	37.8	61.7	69.1	63.7	73.7	56.8	33.6
SO_4	5.0	5.9	7.6	7.8	13.9	13.9	8.0	8.1	8.1	5.4

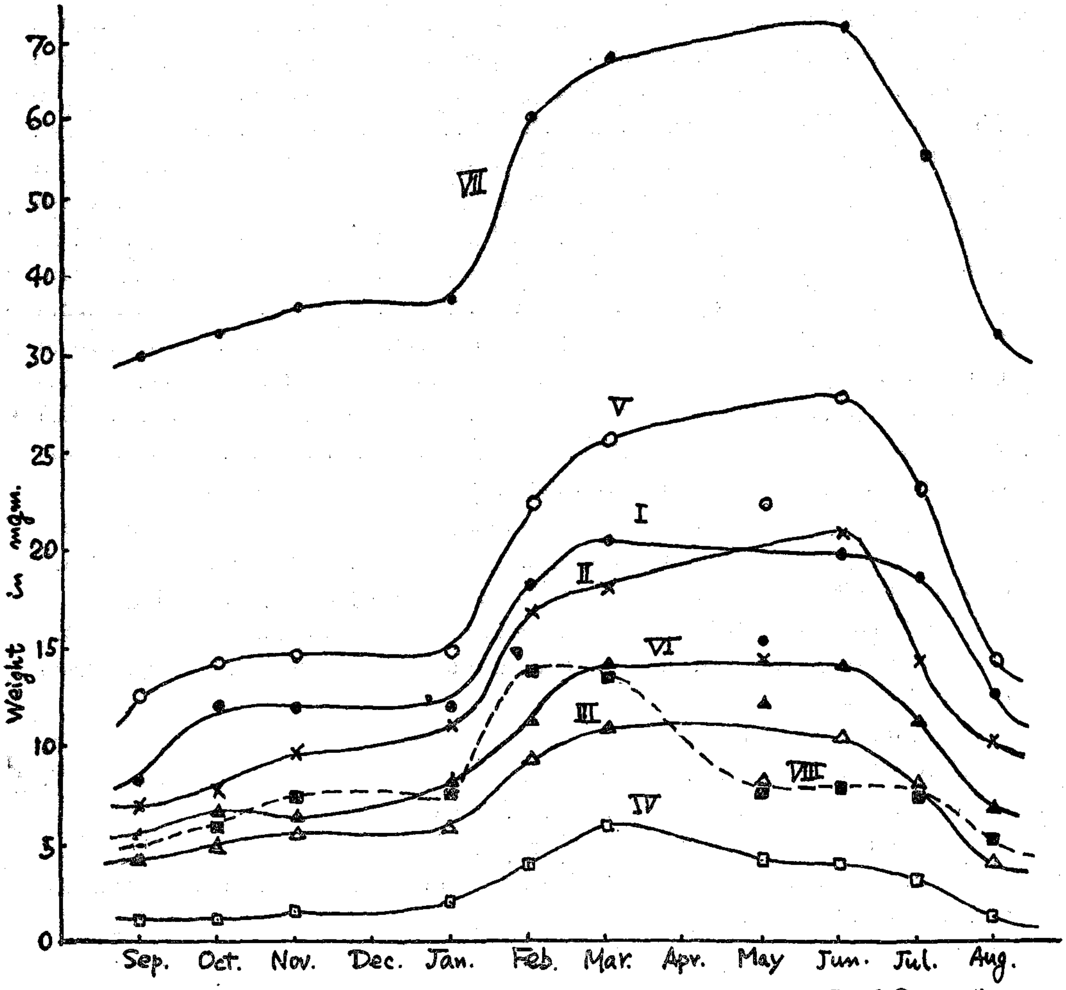

Fig. 7. The Monthly Changes in the Inorganic Constituents of the Pearl Oyster (in Weight). Curve I: Phosphate, II: Calcium, III: Magnesium, IV: Iron, V: Potassium, VI: Sodium, VII: Chloride and VIII: Sulphate.

Table IX.

The Monthly Changes in the Inorganic Constituents of the Pearl Oyster (in Percentage).

	Sep.	Oct.	Nov.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.
	%	%	%	%	%	%	%	%	%	%	%
P_2O_5	13.83	13.86	12.43	11.93	11.37	11.00	10.52	9.52	10.67	12.37	13.71
CaO	9.48	8.80	10.12	10.85	10.47	9.93	10.53	10.21	11.16	10.71	11.26

MgO	5.59	5.70	5.80	5.80	5.87	5.90	5.15	4.98	5.68	5.26	4.32
Fe_2O_3	1.51	1.40	1.65	2.01	2.55	3.27	2.64	2.56	2.28	2.27	1.47
K_2O	16.45	16.37	15.30	14.79	13.28	13.79	13.13	13.81	15.45	14.45	15.48
Na_2O	7.27	7.76	7.32	7.09	7.08	7.65	7.71	7.60	7.58	7.55	7.54
Cl	38.80	37.52	37.95	37.46	38.09	36.78	37.12	39.35	39.00	36.92	35.78
SO_4	6.46	6.75	7.89	7.08	8.56	7.41	6.42	4.94	4.28	5 .24	5.76

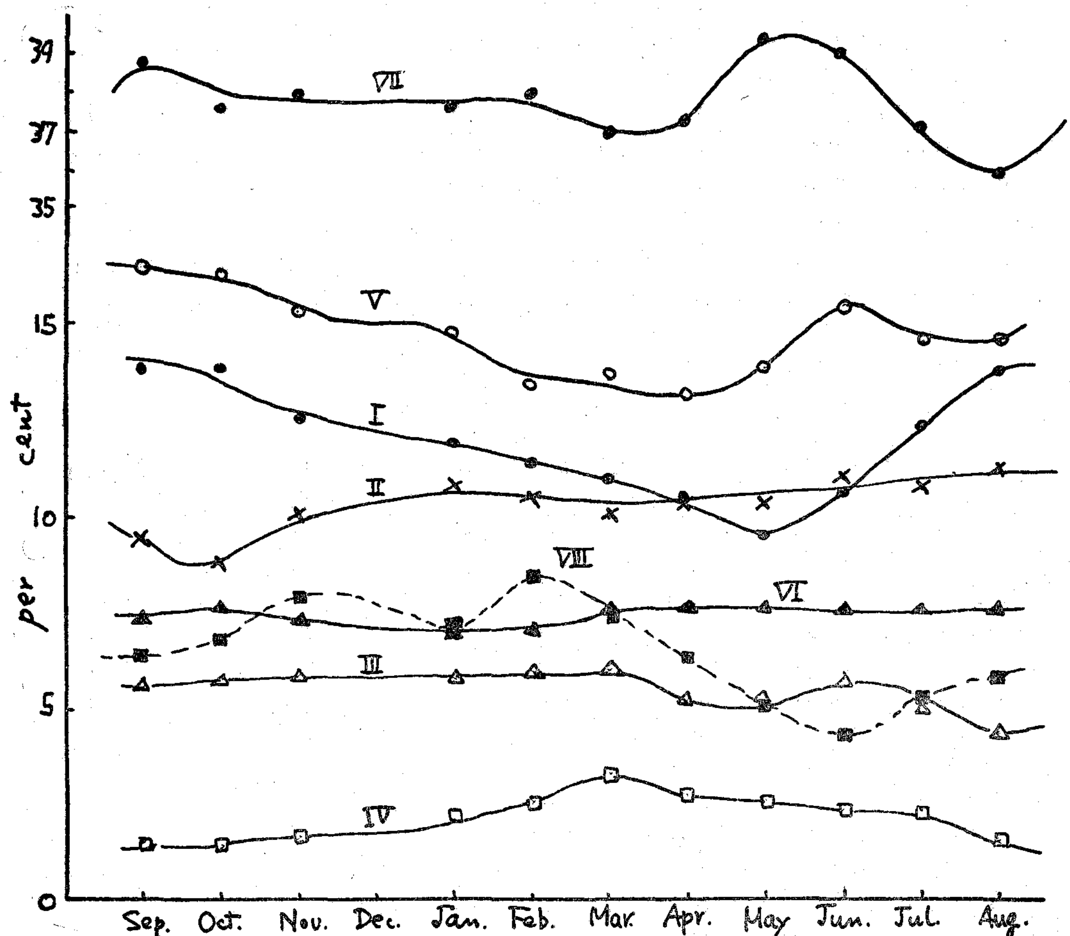

Fig. 8. The Monthly Changes in the Inorganic Constituents of the Pearl Oyster (in Percentage). Curve I: Phosphate, II: Calcium, III: Magnesium, IV: Iron, V: Potassium, VI: Sodium, VII: Chloride and VIII: Sulphate.

Total ash content of the pearl oyster increased from the reproductive period to the beginning of the fattening period. The changes in potassium and phosphate content also agreed with that of the total ash content. These facts suggest that these elements may be related to sexual activity.

From February to March the amount of iron reached a maximum. Since this element has been known as the component of the respiratory enzymes, it may play some significant role in the transformation of certain organic substances such as glycogen and lipids, in the pearl oyster.

It has already been known that taurin, an amino sulphuric acid, is the characteristic substance of the mollusks (9). The content of sulphate may be connected with taurin and other sulphuric esters of carbohydrate (10) which compose the mucus.

Sodium, magnesium and chloride are less variable at the content. The content of calcium in the meat was observed to decrease in the autumn when the shell grew in this season.

From the analytical results of the pearl, mother-of-pearl of the shell and the mucus of the pearl oyster, non-cooperation of phosphate is inferred in the process of calcification of the pearl oyster. (see Table X).

Table X.

Analytical Results of Pearl, Shell and Mucus of the Pearl Oyster

	Pearl (11) (superior)	Pearl (11) (inferior)	Shell (1) (mother-of pearl)
	%	%	%
Water	0.66	0.85	0.64
Total nitrogen	0.72	1.33	0.84
Crude protein	4.50	8.31	5.26
Total ash	92.64	91.85	93.19
SiO_2	0.40	0.51	—
CaO	56.33	54.83	57.00
($CaCO_3$)	(100.4)	(97.7)	(92.92)
MgO	0.97	0.79	—
P_2O_5	trace	trace	trace

Mucus & Mantle Cords(12)	
	%
Total ash	23.31
Calcium oxide	1.45
Magnesium	0.74
Sulphate	2.78
Phosphate	0.62

In stead of cooperation of phosphate in the vertebrate, transportation of calcium at the formation of the pearl and of the shell in the pearl oyster would be carried on with participation of certain proteins of acidic nature in the mucus secreted from mucus cell of mantle cords.

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